

Systematic review of Ethiopian medicinal plants used for their anti-inflammatory and wound healing activities

Article (Accepted Version)

Nigussie, Dereje, Makonnen, Eyasu, Beyene Tufa, Takele, Brewster, Malcolm, Legesse, Belete Adefris, Fekadu, Abebaw and Davey, Gail (2021) Systematic review of Ethiopian medicinal plants used for their anti-inflammatory and wound healing activities. *Journal of Ethnopharmacology*. a114179. ISSN 0378-8741

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Title: Systematic review of Ethiopian medicinal plants used for their anti-inflammatory and wound healing activities

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Abstract

Ethnopharmacological relevance: Plant materials are used worldwide as complementary and alternative therapeutics for the treatment of various illnesses. In Ethiopia, folk medicines are utilized across a wide range of cultures and settings. Ethiopia has numerous plant species of which around 12% are endemic, making it a rich source of medicinal plants that are potentially important for human wellbeing.

Aim of the study: The aim of this study was to assess Ethiopian medicinal plants with anti-inflammatory or wound healing activities, in an attempt to compile the information required for further investigation of their potential role in the management of lymphoedema.

Methods: A systematic review protocol was developed according to the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) statement. The protocol for this review was registered on PROSPERO with registration number CRD42019127471. This review considers all controlled *in vivo* and *in vitro* anti-inflammatory and wound healing studies evaluating the efficacy and safety of Ethiopian medicinal plants. The search strategy included all articles containing descriptors such as Ethiopia, medicinal plants, herbal products, care, management, lymphoedema, lymphedema, swelling, podoconiosis, elephantiasis, wound, wound healing, inflammation, and anti-inflammatory that were published up to June 28, 2019. Outcomes were measured as the percentage of inflammatory and pro-inflammatory cell inhibition, as the percentage of carrageenan-induced oedema (anti-inflammation) inhibition, and the percentage of cell migration and proliferation (wound healing). For quality assessment of individual animal studies, the Risk of Bias tool for animal intervention studies (SYRCLE's RoB tool) criteria was used. For quality assessment of individual *in vitro* studies, the OECD guidelines and the WHO Good Laboratory Practice (GLP) handbook were used.

Results: A total of 46 articles on anti-inflammatory and 17 articles on wound healing properties were reviewed. For the *in vivo* studies, Swiss albino mice and Wistar rats were used, and the concentration of plant extracts or fractions administered to the lab animals varied considerably. Acetone extract of *Vernonia amygdalina* showed the fastest anti-inflammatory activity at lower concentrations in carrageenan-induced paw oedema.

Conclusion: *Lawsonia inermis*, *Azadirachta indica*, *Achyranthes aspera*, and *Cuminum cyminum* are the most studied plant species in terms of anti-inflammatory activity, while *Lawsonia inermis* and *Azadirachta indica* are the most studied for wound healing. The most common *in vivo* techniques used for the anti-inflammatory and the wound healing assays were carrageenan-induced paw oedema, and excision and incision wound models, respectively.

Key words: Ethiopian, Medicinal plants, anti-inflammatory, wound healing, systematic review

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1. Introduction

Traditional herbal medicines are important across a range of societies and continue to be used globally as alternative and complementary treatments for a range of disease conditions. They have immense importance for the health of individuals and communities through the secondary metabolites of the plants that induce biological changes in the body (Mohammad et al., 2013). Presently, there is great interest in medicinal plant research to obtain compounds that have a range of pharmacological activities. Currently, most conventional drugs used for a range of diseases arise from natural sources (Mathew et al., 2008).

Looking for viable and affordable medications, molecules obtained from plants represent an appealing alternative (Pan et al., 2013). Medicinal plants are comparatively non-toxic and are composed of complex molecules with various potential targets and multiple mechanisms of action (Hopkins et al., 2006). Secondary metabolites found in medicinal plants have been reported to have analgesic and anti-inflammatory activities (Mohammad et al., 2013). Most of these metabolites act on cellular processes that cascade inflammation in a similar manner as conventional anti-inflammatory drugs (Joseph et al., 2010). There are also reports of herbal products used for the management of wound healing, which implies that they may be suitable and cheap to use (Mulisa et al., 2015).

The situation is similar in Ethiopia, where traditional medicine is normally established in all societies (Lulekal et al., 2014). In Ethiopia, the practice of traditional medicine has been in place in rural areas for a long time, and many Ethiopian medicinal plants are claimed to have anti-inflammatory and wound healing activities (Gebremeskel et al., 2018). However, data on the safety and efficacy of Ethiopian medicinal plants evaluated for their anti-inflammatory and wound healing activities have not previously been summarized.

Thus, there is a need to investigate the potential therapeutic merits of Ethiopian endemic plants for a range of disease conditions. One of these is lymphoedema, whose management includes reduction of acute attacks and treatment of inflammation and wound infections. This pathological condition is classified into primary or secondary. Primary lymphoedema arises from genetic disorders, and secondary lymphoedema from damage to the lymphatic system, due to lymphatic vessel infestation, lymphadenectomy or radiotherapy in cancer patients (Geroulakos et al., 2007; Lisovoski, 1986). Filariasis is the main cause of tropical lymphoedema, followed by podoconiosis (non-filarial elephantiasis) (Lisovoski, 1986).

Furthermore, recurrent bacterial cellulitis can also result in lymphoedema (Al-niaimi and Cox, 2009).

Inflammation is a complex pathophysiological process mediated by a wide range of signaling molecules produced by leukocytes, macrophages and mast cells as well as by the activation of complement factors resulting in extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site (Mohammed et al., 2014).

Non-steroidal anti-inflammatory drugs are the most widely used medications for the management of pain and inflammation, although they are often associated with adverse effects such as gastrointestinal ulcers and hemorrhage (Bribi et al., 2015). Long-term use of steroidal anti-inflammatory drugs may have severe adverse reactions leading to cardiovascular, endocrine, metabolic, musculoskeletal, and ophthalmologic problems (Chester et al., 2017). Plant materials are claimed to be alternative remedies for the management of pain and inflammation, with fewer side effects (Bribi et al., 2015).

The purpose of managing lymphoedema is to improve lymphatic flow and reduce ‘acute attacks’, which can be achieved through a range of approaches (Jullien et al., 2011). A pharmacological approach is one option to treat acute inflammation and infections, while traditional medicines are widely used for the management of pain and infections (Pan et al., 2013).

Preclinical studies have been conducted on the toxicity and effectiveness of Ethiopian medicinal plants against inflammation and wound healing. However, the effectiveness and toxicity profile of these plants in the management of limb conditions have never been documented.

This systematic literature review aims to summarize information related to Ethiopian medicinal plants found to have anti-inflammatory and/or wound-healing properties in *in vitro* and *in vivo* studies.

2. Methodology

Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines were followed for the conduct and report of this systematic literature review (Moher et al., 2016). The protocol for this review was registered on PROSPERO with registration number CRD42019127471.

2.1. Study design

This review considered pre-clinical studies conducted on anti-inflammatory and wound healing activities of Ethiopian medicinal plants. The component population (disease model), exposure (intervention), comparator and outcome (PICO) of this review are as follows:

Study subjects (disease model) were laboratory animals, and cell lines used for *in vitro* studies. Medicinal plants as whole or their adjuncts: seed, root, flower, bud and leaf extracts used in the experimental groups were considered as interventions. Medicinal plants used were manufactured from single or complex medicinal plants, plant extracts, and plant preparations, regardless of their preparation (extracts, decoctions, tablets, capsules, pills, powders, injections, or other types of preparations), but not synthesized compounds. There were no restrictions on dosage form, concentration, frequency of administration, dose, intensity, or duration of medicinal plants used.

Placebo, vehicle and drugs used for the treatment of controls were considered as comparators. Vehicles were not expected to produce an effect, but helped to identify outside influences on the experiment, such as contamination. Similarly, for placebo (non-intervention), control groups did not receive a treatment or were given an inert substance. The essential outcomes were effectiveness of medicinal plants (at safe concentrations) in reducing symptoms of inflammation in experimental animals, in wound contraction and healing, in comparison with reference drugs. In addition, in *in vitro* studies, down-regulation of inflammatory and pro-inflammatory cells, cell proliferation and migration rate (wound healing) in $\mu\text{g/ml}$ and mg/ml ; minimum effective dose in 50% of the population (ED_{50}), and minimum lethal dose in 50% of the population (LD_{50}). Secondary outcomes were data on liver function tests, organ histopathology results (as a surrogate indicator for long-term toxicity) and mortality.

2.2. Eligibility criteria

Inclusion criteria: Published works in English including theses, articles, and proceedings up to June 28, 2019, that dealt with anti-inflammatory or wound healing activities in *in vivo* or *in vitro* studies.

Exclusion criteria: Newspapers and reviews

2.3.Information sources, searching and selection of studies

Electronic databases such as Scopus, Embase, PubMed/Medline and Google Scholar were used as sources of information for the search. Grey literature such as theses, technical reports, working papers, evaluation reports, conference proceedings, patents, and preprints were also considered for the review. The search technique involved all available articles containing descriptors until June 28, 2019. Only published works written in English were used in this study. Structured search strategies were created using the lexical terms of each database and targeting the “title” and “abstract” fields. Manual searching was also employed using references to previously published works. Search terms such as Ethiopia, medicinal plants, herbal products, care, management, therapeutic, lymphoedema, swelling, podoconiosis, elephantiasis, wound, wound healing, inflammation, anti-inflammatory and other related words or phrases were used.

After looking at the electronic database, all records were transferred to Mendeley referencing software. Studies were pilot-screened before undertaking full study selection. All articles were screened independently by two investigators (DNW and TBT) by skimming the titles and abstracts of the articles based on the criteria. For articles that met the inclusion criteria, investigators read the full document to check the inclusion criteria.

2.4.Data extraction

Two investigators (DNW and TBT) independently extracted data using a data extraction form. Initial calibration exercises were performed to ensure uniformity across the reviewers. The following data were extracted: title, author, year of publication, type of study (*in vivo* or *in vitro*), statistical methods used, type of animals used, age and weight of animals, study duration, number of animals used for the study, route and time of administration; dose/kg, reference drugs used; techniques used to induce inflammation or wounds; concentration at which inflammatory cells were down-regulated or inhibited paw oedema formation; minimum concentration that was toxic to 50% of cells (CC₅₀), type of solvent extracts and fractions used for activity and safety, parts of plant used, extraction type, sources of the plants, place of collection, traditional use, scientific names of the plants, local names of the plants, voucher numbers, types and number of compounds isolated (if any). When separate studies had several treatment groups, the groups were combined to avoid presentation bias caused by different statistical comparisons with one control group (Moher et al., 2016).

2.5.Outcomes measured

For the anti-inflammatory studies on laboratory animals, the key outcomes were the number of animals responding to treatment with the medicinal plant extracts, fractions and compounds, where response was defined as the percentage of carrageenan-induced oedema inhibition and/or the weight of granuloma tissue formation relative to the control group. For the *in vitro* studies of anti-inflammatory activity, the primary outcomes were percent inhibition of inflammatory cells and pro-inflammatory cells, defined as percent inhibition of the enzyme lipoygenase, % inhibition of protein denaturation, levels of inflammatory substances (TNF α , IL6, IL10, and IFN- γ) produced by cells, levels of COX-2 counteracting antibody and levels of nitric oxide (NO) in inflammation-induced cell lines after subsequent treatment with plant extracts. The type of data (variables) extracted for anti-inflammatory and wound healing activities were continuous (percentage and pg/ml, μ g/ml).

In wound healing assays involving lab animals, the key outcomes were percentage shrinkage of wound area, time of epithelization and percentage of tensile strength in experimentally induced wounds, whereas for the *in vitro* wound healing studies, relative cell scattering and movement, percent cell propagation and viability were the key outcomes assessed. Secondary outcomes were long-term effect of plant material, death of animals and experimental dropouts.

2.6. Assessment of risk of bias

Two investigators (DNW and TBT) independently evaluated the risk of bias for each included study. The critical appraisal process for studies involving lab animals was performed using the Risk of Bias tool for animal intervention studies (SYRCLE's RoB tool) and Animal Research: Reporting of *In vivo* Experiments (ARRIVE) guidelines to evaluate the internal soundness of the studies (Connor and Sargeant, 2014; Hooijmans et al., 2014; Kilkenny et al., 2010). A "yes" decision showed a low risk of bias; a "no" decision indicated a high risk of bias; the judgment was considered "unclear" if inadequate details were reported to estimate the risk of bias properly. Studies were evaluated for their internal and external soundness. Reviewers judged the risk of bias for separate elements from five domains of bias (selection, performance, attrition, reporting, and others) using the Cochrane Risk of Bias Tool Appendix D and decided the inclusion and exclusion of the studies (Hooijmans et al., 2014) (Supplementary 1). Then, the risk of bias criteria was referred to as 'low', 'high' or 'unclear'. Studies with a low and moderate risk of bias were reported, whereas a high risk of bias studies was omitted from the analysis.

2.7.Data synthesis

All studies included in the data synthesis were categorized into four separate investigational models as per the kind and aim of the studies: *in vivo* anti-inflammatory, *in vitro* anti-inflammatory, *in vivo* wound healing and *in vitro* wound healing models. Heterogeneity was assessed descriptively from the narrative synthesised data, and potential reasons for heterogeneity were found by examining individual study and subgroup characteristics. Interventional, methodological and statistical heterogeneity existed between the studies, so statistical pooling of studies and meta-analysis could not be performed.

Instead, a narrative (qualitative) summary of the studies was performed using textual descriptions of studies, grouping, and tabulation. Then, a description of the characteristics of the studies compared the effect of each plant extract relative to controls, the main parameters measured/analysed, quality of included studies and the risk of bias of all studies were described. This has also been described in our systematic review protocol (Nigussie et al., 2020).

2.8.Main parameters analysed for *in vivo* and *in vitro* anti-inflammatory assays

The main parameter used to analyse the activity of plant extracts for carrageenan-induced paw oedema (for acute inflammation) was the paw volume. After oral administration of the plant extracts or fractions to the test, reference and control groups, the volume of injected paws was measured before and after the injection of the inflammation-inducing agent (carrageenan) by measuring the linear paw circumference of oedematous legs using a plethysmometer or digital caliper. Alternatively, the volume of oedema was measured using the water displacement method, where the volume of water displaced corresponded to the volume of oedema. Then, the anti-inflammatory activities of plant materials were expressed as percentage and calculated using the following formula:

$$\% \text{ Inflammation (I)} = \frac{V_f - V_i}{V_i} \times 100 \text{ (Tadiwos et al., 2017)}$$

V_i is the paw volume before carrageenan injection and V_f is the volume of paw after carrageenan injection at a given time.

$$\text{Similarly, percent anti - inflammatory} = \frac{\%Ic - \%Ie}{\%Ic} \times 100$$

where %I_c and %I_e are the mean inflammation values reached in the control and experimental groups, respectively.

For sub-acute or chronic inflammation, autoclaved cotton pellets were aseptically implanted subcutaneously in the axillary region of rats anesthetized with diethyl ether. Extracts were administered once daily for 7 days. On day 8, the animals were anesthetized, and cotton pellets were removed surgically, freed from extraneous tissue and dried in an oven overnight at 60 °C. The dried pellets were weighed and the mean weight of granuloma tissue around each pellet was determined. The percent inhibition of granuloma tissue development was calculated using the formula:

$(T_c - T_t)/T_c \times 100$, where T_c = weight of granuloma tissue of control groups T_t = weight of granuloma tissue of treated groups (Sharma and Rajani, 2011).

Many methods were used to measure the anti-inflammatory activity of plant material in the *in vitro* model. The main parameters measured in the studies included were percent inhibition of production of cyclooxygenase enzymes (COX-1 and COX-2), inflammatory biomarkers such as vascular cell adhesion molecule-1 (VCAM-1), interferon gamma-induced protein 10 (IP-10), interferon-inducible T-cell and chemoattractant (I-TAC), and monokine induced by interferon (MIG), nitric oxide (NO) production, IL-1 β and IL-6 mRNA expression; quantity of pro-inflammatory cytokines (TNF α and IL-6), median inhibitory concentration (IC₅₀) values, percent inhibition of protein denature and gene expression of inflammatory cells.

2.9. Main parameters analysed for the *in vivo* and *in vitro* wound healing assay

The main parameters measured in the *in vivo* wound healing studies were percentage wound contraction, histopathological studies (collagen formation, fibroblast proliferation and angiogenesis), percent tensile wound strength (skin breaking strength), period of epithelization (number of days required for complete falling of scab without any residual raw wound) after the treatment of incision and excision wounds with plant extracts and standard drugs. The parameters were calculated using the following formulae:

% Wound contraction = (Healed area (mm²))/ (Original wound area) *100

Where healed area = original wound area – present wound area.

% Tensile strength (TS) of test sample = ((TS of test sample- TS so))/ (TS so) x 100

% Tensile strength (TS) reference = ((TS of reference-TS So))/ (TS So) x100

% Tensile strength (TS) of So = ((TS So-TS Lu))/ (TS Lu) x 100

where, So and Lu stands for simple ointment treated and left untreated groups, respectively.

Several methods were used to measure the outcomes of wound healing. For wound contraction, wound areas were measured at different time intervals using a transparent sheet and a permanent marker. The period of epithelization was measured by the number of days required for sloughing of the dead tissue, and the tensile strength of the wounds was measured using the continuous water flow method. Histopathological characteristics such as hydroxyproline content, content of collagen formation, fibroblast proliferation and angiogenesis were measured.

For the tests and controls, plant extracts and standard drugs were applied until the wound healed (for excision wounds) or for 10 to 11 days (for incision wounds). Measurement of treatment effect (end point) of wound contraction was conducted on a daily basis for 10 - 11 days, while the period of epithelization and tensile strength were measured on the 10th and 11th days (most studies) or every other day (in some studies) after the application of plant extracts and standard drugs.

The main parameters measured in the *in vitro* wound healing assay were cell migration and proliferation and IC₅₀ values for radical scavenging activity. Cytotoxicity assays were conducted for all medicinal plants, and all concentrations used for activity were safe to the cell lines used for the tests. In the first study, aqueous extracts of seventeen medicinal (Hooijmans et al., 2014) plants were studied using a wound healing scratch assay in 3T3 fibroblasts cell lines. The outcome measured after 21h of exposure to test and reference substances was cell migration and proliferation using a Leica DMLS microscope at 4x magnification before and after incubation to estimate the proliferation and migration of cells. Then, cell proliferation/migration rate was calculated as percent closure of the linear scratch made within 21h, as shown below:

$$\frac{(\text{Cell migration})}{(\text{Cell proliferation})} = \frac{((\text{gap distance } t_0 - \text{gap distance } t_{21}))}{(\text{gap distance } t_0)} \times 100$$

t_0 = at time zero, t_{21} = time after 21 hr

2.10. Quality of studies in vivo and in vitro anti-inflammatory assay (bias analyses)

The critical appraisal process for *in vivo* anti-inflammatory activity was performed using the Risk of Bias tool for animal intervention studies (SYRCLE's RoB tool) and ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines to assess the validity of scientific findings (Hooijmans et al., 2014; Kilkenney et al., 2010). Studies with high levels of bias were excluded. However, the studies included still have some weaknesses in their methodology. External and internal validity of the studies were evaluated. In external validity evaluation using animals as models for basic biological functions, the implication is that the results could be extrapolated to humans (Connor and Sargeant, 2014).

We categorized the judgment of bias as yes, no or unclear. A "yes" judgment indicated low risk of bias; a "no" judgment indicated high risk of bias; the judgment was "unclear" if insufficient details had been reported to assess the risk of bias properly.

Under internal validity, the following ten main criteria were used: (1) adequate generation and application of the allocation sequence, (2) similarity of the control and test groups at baseline, (3) adequate concealment of allocation to different groups, (4) random housing of the animals during the experiment, (5) blinding caregivers and/or investigators to knowledge of which intervention each animal received during the experiment, (6) random selection of animals for outcome assessment, (7) blinding the outcome assessor, (8) adequate addressing of incomplete outcome data, (9) outcome reports from selection bias, and (10) absence of high risk of bias. The critical appraisal process for *in vitro* anti-inflammatory activity was performed using the Guidance Document on Good *In Vitro* Method Practices (GIVIMP) *in vitro* Studies (CRIS) guidelines.

For the *in vitro* studies, quality assessment was based on seven main criteria which were extracted from these guidelines: (1) quality assurance of all materials and methods, and their use and application, in order to maintain the truthfulness, soundness and reproducibility of laboratory work conducted; (2) appropriateness of the *in vitro* cell and tissue culture facilities for purpose and a detailed understanding of the work flow for the *in vitro* methods and related processes; (3) apparatus, materials and reagents; (4) cell lines, media and serum, (5) test substance and reference/control items, (6) performance of the experiment, and (7) data analysis and interpretation. Under each criterion there are 2-8 sub criteria to evaluate the internal validity of the studies.

The judgment of bias was categorized as yes, no or unclear. A “yes” judgment indicated low risk of bias; a “no” judgment indicated high risk of bias; judgment was “unclear” if insufficient details were reported to assess the risk of bias properly.

2.11. Quality of *in vivo* and *in vitro* wound healing studies (bias analyses)

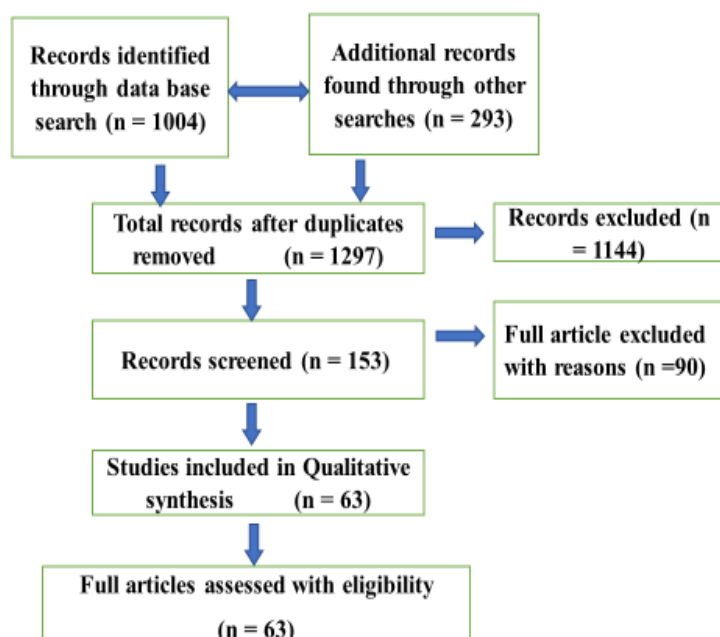
The critical appraisal checklist we used for *in vivo* wound healing studies was similar to the *in vivo* anti-inflammatory activity. The Risk of Bias tool for animal intervention studies (SYRCLE’s RoB tool) and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were used to assess the external and internal validity of the studies. Similarly, the judgment of biases was categorized as yes, no or unclear (Connor and Sargeant, 2014; Kilkenny et al., 2010). The quality of these two studies was evaluated using the method and guidelines used *in vitro* anti-inflammatory studies. The Guidance Document on Good In Vitro Method Practices (GIVIMP) (Antonelli et al., 2018), and the Checklist for Reporting In vitro Studies (CRIS) guidelines (Krithikadatta et al., 2019), were used for the internal quality test of the studies. As above, the quality of the studies was evaluated based on seven main criteria. The judgment of biases was categorized as ‘yes’, ‘no’ or ‘unclear’ under each criterion; and based on this judgment, studies were either included or excluded in data extraction.

3. Results

3.1.Literature search results and description of study characteristics

A total of 1297 relevant articles were independently identified by two reviewers for preliminary review from electronic and manual searches. Of these, 649 were from PubMed/Medline, 314 from Embase, 38 from Scopus, 3 from Google Scholar, and 293 from other sources. After removal of duplicates by reviewing relevant titles and abstracts, a total of 153 articles were retrieved for full text review. After a detailed review of each article, 90 articles were excluded and 63 articles were retrieved: *in vivo* anti-inflammatory (n=31), *in vitro* anti-inflammatory (n=15), *in vivo* wound healing (n=15), and *in vitro* wound healing (n=2) studies (Figure 1).

Figure 1 - Flow Diagram of the *in vivo* and *in vitro* anti-inflammatory and wound healing literature search results



3.2.Excluded studies

Many studies have been conducted in these areas. However, most of the articles were not eligible for inclusion. The reasons for exclusion are given as follows:

- Incomplete information:
 - ✓ The concentration of plant extracts used for activity and the number of experimental duplicates were not reported.
 - ✓ The method of outcome measurement was not reported.
 - ✓ Negative and positive controls used were not reported.
 - ✓ The time at which the outcomes were measured, sources of the cell lines and statistical methods used for data analysis were not reported.
- Not relevant studies:
 - ✓ Clinical trial studies;
 - ✓ Studies conducted on medicinal plants that are not growing in Ethiopia.
 - ✓ Activity was not conducted for human pathogens (animal and plant pathogens).

3.3. Included studies

3.3.1. *In vivo* anti-inflammatory studies

Characteristics of the studies

Thirty-one studies met the review criteria, and the year of publication of the studies ranged from 1994 to 2019. Thirty were peer-reviewed full articles, and one was an MSc thesis. All study designs met the criteria for inclusion and followed internationally accepted guidelines. Studies were conducted in eight different countries. These were Ethiopia (n=13), Austria (n=1), Costa Rica (n=1), Egypt (n=3), India (n=5), Iran (n=1), Nigeria (n=4) and Tunisia (n=2). Thirty-five (35) plant species were investigated for anti-inflammatory activity in animal models (Table 1). All the medicinal plants investigated were found in Ethiopia, and they were investigated for anti-inflammatory, analgesic, antioxidant, anti-microbial, wound healing, anti-ulcer and/or anti-arthritic activities in animal models.

The following plant families were reported in the studies of *in vivo* anti-inflammatory assays. These were *Oleaceae*, *Lythraceae*, *Amaranthaceae*, *Meliaceae*, *Lamiaceae*, *Lauraceae*, *Asteraceae*, *Malvaceae*, *Myrtaceae*, *Apiaceae*, *Anunculaceae*, *Fabaceae*, *Zingiberaceae*, *Combretaceae*, *Acanthaceae*, *Polygonate*, *Salicaceae*, *Adiantaceae*, *Anacardiaceae*, *Rosaceae*, *Xanthorrhoeaceae*, *Cucurbitaceae* and *Moringaceae* (Table 1). All the plants investigated were authenticated by botanists and had voucher numbers. The most studied plant parts were leaves (n=16), followed by aerial parts (8), roots (n=3), rhizomes (3), fruits (n=3), stem bark (n=2) and whole plants (n=2). The most frequently used techniques for the extraction of medicinal plant parts were maceration (n=20), followed by Soxhlet (n=6) and decoction (n=5) methods. The rest of the investigators used other methods and two combined methods (maceration and Soxhlet) together. Methanol (n=11) was the most frequently used solvent for crude extraction, followed by water (n=7), ethanol (n=6) and acetone (n=1).

Most studies used both sexes of Swiss albino mice (n=15) and Wistar rats (n=16). The age ranges of Swiss albino mice and Wistar rats used for the experiments were 6-8 weeks and 12 - 13 weeks, respectively.

There was a wide range of variation in the concentration of plant extracts or fractions administered to the animals. Dose selection was made based on acute toxicity tests and pilot experiments. All concentrations used were safe doses to the laboratory animals and most

extracts were administered orally to the animals. On the other hand, (Naik et al., 2014), (Bhosale et al., 2012), (Mohammed et al., 2014), (Hosseinzadeh et al., 2011) and (Badilla et al., 1999) used intra-peritoneal and subcutaneous routes to administer the extracts to the animals, while (Khedir et al., 2016) used topical route of application (Supplementary table 1).

Studies also used carrageenan-induced mouse paw oedema model to check the anti-inflammatory activities of different extracts at three different doses, 100, 200, and 400 mg/kg (Tadiwos et al., 2017; Abdissa, 2011; Wolde-Mariam et al., 2013; Ayal and Belay, 2019; Ching et al., 2009; Alemu et al., 2018).

In another study, Hamad et al., (2019) and Gebremeskel et al., (2018) used 200, 400 and 600 mg/kg doses for different plant extracts. However, Yonathan et al., (2006) used 400 mg/kg dose, Dilebo et al., (2011) used 300 and 500 mg/kg doses, Mulisa et al., (2015) used 250, 500 and 750 mg/kg doses; 50, 150 and 350 mg/kg doses. Three studies, Adedapo et al., (2014), Masresha et al., (2012) and Mequanint et al., (2011) used 400, 600, and 800 mg/kg doses. However, Sewuye and Asres (2009) used a 200 mg/kg dose. (Supplementary table 1).

Similarly, cotton pellet-induced Granuloma method in mice was used to measure chronic inflammation. Umer et al., (2014) and Alemu et al., (2018) used 100, 200, and 400 mg/kg doses of 80% methanol extracts, However, Hosseinzadeh et al., (2011) used 5, 15, 30, 100, and 200 mg/kg aqueous extract and 0.05, 0.15, and 0.35 g/kg ethanolic extracts for chronic inflammation. Vijayaraj and Kumaran (2018) and Sharma and Rajiani (2011) used 100 and 200 mg/kg doses of ethanol and water extracts (Supplementary table 1).

Aspirin, diclofenac, indomethacin and dexamethasone were used as standard drugs. The number of animal groups for anti-inflammatory activities depended on the number of different concentrations tested. The number of groups ranged from 3 to 5, where six animals were randomly assigned to each group.

For the carrageenan-induced paw oedema method, the duration of exposure of the animals to the extracts (treatment) was 4-6hrs, and the time of measurement of end points was after 1, 2, 3, 4, 5, and 6 hrs of exposure.

At each end point the volume of paw oedema of the test and control groups was measured, and the percentage inhibition of oedema formation was calculated. For the cotton pellet-induced granuloma method (chronic inflammation), animals were exposed to treatment for 7 to 14 days and end points were measured on the 8th and 15th days; weight of cotton pellet (gm), granulation

tissue formation (gm) and percent inhibition of tissue granulation were the end points measured. Seventeen studies used one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, and six used the Student t-test.

Most studies used carrageenan-induced paw oedema (n=23) to study the anti-inflammatory activity of the plant materials in laboratory animals, while some of them used other methods such as topical croton oil-induced or xylene-induced ear oedema method, histamine-induced paw oedema, and serotonin-induced paw oedema.

Except for Dilebo et al., (2011), all studies showed at least one safe concentration of the plant material with significant inhibition of inflammation in animals in a dose-dependent manner. The maximum inhibition of oedema at low doses was reported by Tamrat et al., (2017) which showed 64.71%, 76.47% and 82.35% inhibition, respectively, at 50, 100, and 200 mg/kg of the methanol extract of *J. abyssinicum* 2h after carrageenan injection (Tadiwos et al., 2017).

Aqueous extract of *Achyranthes aspera* exhibited higher inhibition of paw oedema formation at a dose of 800 mg/kg compared with vehicle control in mice in 4h (Bhosale et al., 2012). *Azadirachta indica* chloroform extract was shown to have 53.25% inhibition of oedema in rats, comparable to the control drug indomethacin (46.9%) (Umar et al., 2014). Fraction-2 from *Azadirachta indica* showed higher inhibition of granuloma tissue formation at doses of 200 and 400 mg/kg in rats. The activity of a flavonoid isolated from *Lawsonia inermis* was similarly reported by (Manivannan and Aeganathan, 2015) to have significant anti-inflammatory activity at doses of 100 and 200 mg/kg in rats after induction of inflammation in 3h.

Acetone extract of *Vernonia amygdalina* (Gerawa) showed the fastest activity at low concentrations (Adedapo et al., 2014). They reported that 100 mg/kg and 200 mg/kg doses of the extract reduced inflammation after 1h, 2h and 3h incrementally compared to the controls. The 200 mg/kg dose of the extract gave the most pronounced inhibition of oedema after 3h, which was greater than that of the standard drug, indomethacin (Adedapo et al., 2014). On the other hand, Dilebo et al., (2011) reported that the methanol extract of *Ferula communis* (Doge) was devoid of mouse paw oedema inhibition. The highest dose of plant extract used was reported by Adeyem et al.(2002), which showed that the aqueous extract of *Persea Americana* (avocado) significantly ($p<0.05$) inhibited carrageenan-induced rat paw oedema at 1600 mg/kg, similar to that produced by 100 mg/kg of acetylsalicylic acid with percent inhibition of 57.2% and 58.0%, respectively (Adeyemi et al., 2002).

520 In another study, aqueous extracts of different parts of *Citrullus colocynthis* (roots, stems, fruits
521 and seeds) injected intraperitoneally at a dose of 1 mg/kg and 4 mg/kg showed moderate to
522 high activity (Marzouk et al., 2010). In this study, immature fruits (88.33%) and seeds
523 (98.06%) of *Citrullus colocynthis* showed better activity than ripe fruits (65.00% to 97.90%).
524 The percent inhibition of aqueous extracts of the root and stem of *Citrullus colocynthis* was
525 90.00% and 98.84%, respectively, showing better activity compared to the standard drug,
526 acetyl salicylate of lysine (74.40% after 4h) (Marzouk et al., 2010).

527 Three studies were included for the evaluation of chronic inflammation (cotton-pellet). The
528 first study was reported by Alemu et al., (2018) who used 100, 200, and 400 mg/kg doses (oral)
529 of *Leonotis ocymifolia* 80% methanol extract, and showed significant ($p<0.001$) inhibition of
530 the formation of inflammatory exudates and granuloma mass. On the other hand, 28.91%,
531 37.68%, and 45.91% inhibition of inflammatory exudate and 24.03%, 40.18%, and 50.65%
532 reduction of granuloma were recorded at 100 mg/kg, 200 mg/kg, and 400 mg/kg doses,
533 respectively, while, dexamethasone showed 52.89% and 79.54% inhibition of exudates and
534 granuloma, respectively (Alemu et al., 2018).

535 The second study used different concentrations of aqueous (0.005, 0.015, 0.03, 0.1, and 0.2
536 g/kg) and ethanol (0.05, 0.15, and 0.35 g/kg) extracts of the aerial part of *Myrtus communis*
537 (Hosseinzadeh et al., 2011). The aqueous extract exhibited significant inhibition of
538 inflammation in a dose-dependent manner. Maximum efficacy (57.9%) was noted for a dose
539 of 0.03 g/kg of the aqueous extract (Hosseinzadeh et al., 2011). The last study was on the anti-
540 inflammatory activity of ethanol and aqueous extracts of *Caesalpinia pulcherrima* (aerial) in a
541 rat model. They used 100 mg/kg and 200 mg/kg doses of both ethanol and aqueous extracts
542 orally. Both extracts showed a significant ($p<0.05$) inhibition of the growth of granuloma
543 tissues compared to the controls. A water extract of 200 mg/kg dose ($54.09\pm2.76\%$) produced
544 significant ($p<0.05$) inhibition of granuloma tissue compared to indomethacin ($53.32\pm9.00\%$).
545 The aqueous extracts had better anti-inflammatory efficacy than the ethanol extracts (Sharma
546 and Rajani, 2011).

547 Generally, the titles of the *in vivo* anti-inflammatory studies that were analysed were consistent
548 with the objectives that the authors stated. Overall, the risk of performance and selection biases
549 was low because studies adequately generated and applied the allocation sequence, and
550 allocations were adequately concealed. Furthermore, the baseline characteristics of the
551 different treatment groups did not significantly differ within any of the included studies.

However, eighteen studies did not report whether animals were randomly housed during the experiment (risk of performance bias) or not.

In addition, not all studies reported whether the animal caregivers or investigators were blinded during the experiment (risk of performance bias). Similarly, it is unclear whether the outcomes were assessed blindly or not (detection bias). Five studies did not report whether all animals were included in the analysis (risk of attrition bias). Apparently, all studies followed the study protocol and were free of selective reporting outcomes.

3.3.2. *In vitro* anti-inflammatory studies

Characteristics of the studies

For the *in vitro* anti-inflammatory activity, fifteen studies were included for full review and data extraction. The year of publication ranged from 2011 to 2019 and all of them were peer-reviewed full articles. Like the *in vivo* anti-inflammatory studies, all the study designs met the inclusion criteria and followed internationally accepted guidelines/protocols. Studies were conducted in South Africa (n=1), USA (2), India (n=3), China (3), Canada (n=1), Tunisia (n=1), Morocco (n=1), Nigeria (n=1), Luxembourg (n=1), and Cameroon (n=1). However, no study was conducted in Ethiopia though the medicinal plants investigated can all be found in Ethiopia. The studies investigated the antioxidant, anti-granuloma and anti-bacterial activities, and anti-acetylcholinesterase activity.

A total of 23 medicinal plant extracts were investigated (Table 1). Two of the medicinal plants, commonly used in Chinese traditional medicine (*Zingiber officinale* and *Artemisia annua*), are also known for their medicinal use in Ethiopia. Plant parts such as leaves (n=7), seeds (n=5), whole plants (n=3), roots (n=2), bark, stems (n=1), fruits (n= 1), flowers (n=1) and bulbs (n=1) were investigated in these studies.

Plant families of the plants reported in this review were *Apocynaceae*, *Myrtaceae*, *Berberidaceae*, *Zingiberaceae*, *Apiaceae*, *Betulaceae*, *Meliaceae*, *Amaranthaceae*, *Brassicaceae*, *Cucurbitaceae*, *Rosaceae*, *Anacardiaceae*, *Lythraceae*, *Lamiaceae*, *Myrtaceae*, *Solanaceae*, *Fabaceae*, *Poaceae*, *Verbenaceae*, *Rutaceae*, *Liliaceae*, *Euphorbiaceae* and *Capparidaceae* (Table 1).

For the *in vitro* anti-inflammatory assays, commercially available cell lines and mononuclear cells (macrophages) isolated from laboratory animals were used. These were; Primary human

neonatal fibroblasts (HDF3CGF assays/ELSEA), RAW264.7 cells lines (for nitric oxide assay), RAW 264.7 cell lines (cytokines assay, Real time PCR and Western blotting), peritoneal mice macrophages (cytokines assay, western blotting), HP-1 cells lines (TNF-alpha assay), Murine macrophages RAW264.7 cells, mouse splenocytes, and human embryonic kidney 293 (HEK293) cell lines, primary human neonatal fibroblasts (gene expression assay) and kits for colorimetric *in vitro* anti-inflammatory assay were used.

Cytotoxicity assays were conducted for all plant extracts tested in the *in vitro* models and all concentrations used were safe to the cell lines used in the experiments. The concentration of essential oils used for *in vitro* anti-inflammatory activity ranged from 0.00041- 0.011% (v/v).

Han and Parker, (2017) tested 0.011, 0.0037, 0.0012, and 0.00041% (v/v) of the essential oil of *Eugenia caryophyllata*, and noted that they significantly decreased the levels of inflammatory biomarkers such as vascular cell adhesion molecule-1 (VCAM-1), interferon gamma-induced protein 10 (IP-10), interferon-inducible T-cell a chemoattractant (I-TAC), and monokine induced by c interferon (MIG). This indicated that *Eugenia caryophyllata* essential oil may possess anti-inflammatory and pro-wound-healing properties. The effects of *Eugenia caryophyllata* essential oil on these biomarkers were concentration-dependent (Han and Parker, 2017a) (Supplementary table 1).

Methanol extracts of *Lawsonia inermis* showed cyclo-oxygenase enzyme inhibition (IC₅₀ value of 51 ± 0.23 mg/L), and inhibition of heat induced albumin denaturation and hemolysis (IC₅₀ = 170.24 µg/ml) (Chaibi et al., 2017; Bouhlali et al., 2016). Similarly, *Rosa damascena* flower extract exhibited the highest inhibition of haemolysis (IC₅₀ = 125.02 µg/ml). Fraction-2 (F-2) of chloroform extract of *Azadirachta indica* inhibited the production of IL-1 and TNF-α in a dose dependent manner. Fractions 1, 2 and 3 had significant activity on COX-1 and COX-2 at 200 µg/ml, comparable to indomethacin.

In another study, 0.01% (v/v) essential oil of *Cuminum cyminum* inhibited iNOS and COX-2 mRNA levels in Lipopolysaccharide (LPS)-stimulated cells; and reduction of proinflammatory cytokines was observed in a dose-dependent manner. *Cuminum cyminum* essential oil (0.01%) reduced IL-1β and IL-6 mRNA expressions to 30.2% and 1.3% in LPS-stimulated cells (Wei et al., 2015). Furthermore, 0.01% *Cuminum cyminum* essential oil produced 52% inhibition of nuclear NF-kB p65 levels in LPS-stimulated cells, which exhibited anti-inflammatory effects partially by blocking NF-kB activation; it also resulted in 45% and 53% inhibition of LPS-

induced JNK and ERK phosphorylation which was partially attributed to regulating JNK and ERK pathways (Wei et al., 2015) (Supplementary table 1).

Essential oils of *Allium sativum*, *Allium cepa*, *Drypetes gossweileri*, *Pentadiplandra brazzeana* were also tested and inhibited the denaturation of protein (albumin) in a concentration dependent manner (3.125 to 125 µg/ml) (Ndoye Foe et al., 2016). In addition, essential oil of *Cymbopogon flexuosus* (lemon grass) significantly decreased production of several inflammatory biomarkers, including vascular cell adhesion molecule 1 (VCAM-1), interferon gamma-induced protein 10 (IP-10), interferon-inducible T-cell alpha chemoattractant (I-TAC), and monokine induced by gamma interferon (MIG) (Han and Parker, 2017) (Supplementary table 1).

The time of measuring the outcomes for the essential oils varied significantly across the experiments, i.e., 24h (n=4), 15 minutes (n=1) 10 minutes (n=1) and 30 minutes (n=1).

The concentration of plant extracts reported ranged from 0.25 µg/ml to 100 µg/ml. The minimum concentration reported for *in vitro* anti-inflammatory activity was 0.25 µg/ml for dichloromethane and ethanol extracts of *Huernia hystrix*. In this study, petroleum ether extract of roots, all the petroleum ether and dichloromethane extracts showed good activity by inhibiting cyclo-oxygenase enzyme 1 and 2 (COX-1 and COX-2) by more than 70% (Amoo et al., 2012). The highest concentration of methanol extract of *Alnus nepalensis*, used for the test was 100 µg/ml (Saxena et al., 2018). The *in vitro* anti-inflammatory activity of the leaf extract of *Alnus nepalensis* against pro-inflammatory cytokines (TNF-α and IL-6) were assessed using ELISA technique in LPS-induced inflammation macrophage cells at concentrations of 10, 30 and 100 µg/ml. *Alnus nepalensis* butanol fraction significantly inhibited the production of pro-inflammatory cytokines (TNF- α and IL-6) in a dose-dependent manner (Saxena et al., 2018).

In another study, anti-inflammatory activity of twelve different medicinal plants was investigated. *Amaranthus dubius* was reported to be the most potent extract with IC₅₀ of 9 µg/ml, followed by *Ocimum americanum*, *Vigna unguiculata*, and *Zanthoxylum chalybeum* with IC₅₀ values of 16, 27, and 47 µg/ml respectively. However, *Brassica oleracea*, *Ocimum gratissimum*, *Azadirachta indica* and *Mangifera indica* showed weaker dose responses (Tufts et al., 2015).

Generally, all *in vitro* anti-inflammatory studies included fulfilled the test definition, which included the purpose, requirements and scientific basis of the tests. In addition, all the methods followed were valid, internationally acceptable, clearly written and documented *in vitro*

method descriptions. Relevant documentation of proof of sterility, laboratory consumables suitability and acceptability for use was reported in all studies. However, none of the studies reported evidence of provision of relevant and adequate education and training for all personnel to maintain high quality work and safety.

Furthermore, no studies reported whether the *in vitro* cell and tissue culture facilities were fit for purpose, or a detailed understanding of the workflow for the *in vitro* methods, which may adversely affect the quality of the work performed. One study reported the compliance of laboratory suppliers with good laboratory practice (GLP) principles, whereas nine of them did not report this. Reference data to assess the relevance of *in vitro* methods were provided; sources, components and batch numbers of media and serum used were precisely specified; and maximum acceptable levels of serum components were defined well.

In addition, reference and control items, concentration of solvent(s) used, suitability of reference and control items, and justification for the selection of the reference item(s) were reported in all the studies. However, eight studies did not report the applicability domains of the *in vitro* methods, and the limitations of the test methods were not described well (risk of performance bias).

The number of replicates for each testing condition, the concentration level(s) used for the test and control items and the measurement of the outcomes were uniform across the whole plate (well-to-well), between plates and across multiple runs which minimised any potential systematic effects. Appropriate statistical methods were used for all *in vitro* anti-inflammatory studies.

Table 1. Summary of common medicinal plants investigated as anti-inflammatory agents from literature search

S/N	Plant species	Family	Life form	Part s used	Number of citations	Ref.
1	<i>Jasminum abyssinicum</i>	Oleaceae	Herb	R	1	(Tadiwos et al., 2017)
2	<i>Leonotisocymifolia</i>	Lamiaceae	Shrub	L	1	(Alemu et al., 2018)
3	<i>Persea americana</i>	Lauraceae	Tree	L	1	(Adeyemi et al., 2002)
4	<i>Bidens Pilosa</i>	Asteraceae,	Herb	L	1	(Dilebo et al., 2011)
5	<i>Malva verticillate</i>	Malvaceae,	Herb	L	1	(Dilebo et al., 2011)
6	<i>Syzygium guineens</i>	Myrtaceae,	Tree	L	1	(Dilebo et al., 2011)
7	<i>Ferula communis</i>	Apiaceae,	Herb	Rh	1	(Dilebo et al., 2011)
8	<i>Ranunculus multifidus</i>	Ranunculaceae	Herb	Ae	1	(Dilebo et al., 2011)
9	<i>Stereospermum kunthianum</i>	Bignoniaceae	Shrub	Sb	1	(Ching et al., 2009)

10	<i>Melilotus elegans</i>	Fabaceae	Herb	L	1	(Asres et al., 2005)
11	<i>Urera baccifera</i>	Urticaceae	Shrub	L	1	(Badilla et al., 1999)
12	<i>Curcuma longa</i>	Zingiberaceae	Herb	Rh	1	(Mohamed et al., 2013)
13	<i>Cinnamomum xylanicum</i>	Lauraceae	Herb	B	1	(Mohamed et al., 2013)
14	<i>Thyme vulgaris</i>	Lamiaceae	Herb	Ae	1	(Mohamed et al., 2013)
15	<i>Combretum aculeatum</i>	Combretaceae	Tree	Ae	1	(Hamad et al., 2019)
16	<i>Myrtus communis</i>	Myrtaceae	Shrub	Ae	1	(Hosseinzadeh et al., 2011)
17	<i>Vernonia amygdalina</i>	Asteraceae	Shrub	L	1	(Adedapo et al., 2014)
18	<i>Aniso testrisulcus</i>	Acanthaceae	Herb	Ae	1	(El-Shanawany et al., 2014)
19	<i>Calpurnia aurea</i>	Fabaceae	Shrub	L	1	(Ayal and Belay, 2019)
20	<i>Caesalpinia pulcherrima</i>	Fabaceae	Shrub	Ae	1	(Sharma and Rajani, 2011)
21	<i>Diclipter alaxata</i>	Acanthaceae	Herb	Ae	1	(Wolde-Mariam et al., 2013)
22	<i>Rumex abyssinicus</i>	Polygonaceae	Herb	Rh	1	(Mulisa et al., 2015)
23	<i>Dovyalis abyssinica</i>	Salicaceae	Shrub	F	1	(Abdissa, 2011)
24	<i>Ocimum lamiifolium</i>	Lamiaceae	Herb	L	1	(Mequanint et al., 2011)
25	<i>Cheilanthes farinosa</i>	Adiantaceae	Shrub	Ae	1	(Yonathan et al., 2006)
26	<i>Pistacia lentiscus</i>	Anacardiaceae	Shrub	F	1	(Khedir et al., 2016)
27	<i>Ocimum suave</i>	Labiatae	Herb	L	1	(Masresha et al., 2012)
28	<i>Rosa abyssinica</i>	Rosaceae,	Shrub	R	1	(Sewuye and Asres, 2009)
29	<i>Salvia nilotica</i>	Lamiaceae	Shrub	L	1	(Sewuye and Asres, 2009)
30	<i>Aloe megalacantha</i>	Xanthorrhoeaceae	Shrub	L	1	(Gebremeskel et al., 2018)
31	<i>Citrullus colocynthis</i>	Cucurbitaceae	Herb	R, S, F	1	(Marzouk et al., 2010)
32	<i>Moringa stenopetala</i>	Moringaceae	Tree	L	1	(Tamrat et al., 2017)
33	<i>Lawsonia inermis</i>	Lythraceae	Tree	L	6	(Annabarapu et al., 2016; Bouhlali et al., 2016; Vijayaraj and Kumaran, 2018; Chaibi et al., 2017)
34	<i>Azadirachta indica</i>	Meliaceae	Tree	L	3	(Annabarapu et al., 2016, Nik et al., 2014, Pereira et al., 2012, Schumacher et al., 2011, Tftus et al., 2015, Umar et al., 2014)
35	<i>Achyranthes aspera</i>	Amaranthaceae	Herb	L	2	(Bhosale et al., 2012; Gokhale et al., 2002)
36	<i>Huernia hystrix</i>	Apocynaceae	Herb	S, R, W	1	(Amoo et al., 2012)
37	<i>Eugenia caryophyllata</i>	Myrtaceae	Tree	S	1	(Han and Parker, 2017a)
38	<i>Zingiber officinale Rosc</i>	Zingiberaceae,	Herb	W	1	(Chen and Zhang, 2014)

39	<i>Artemisia annua</i>	Asteraceae,	Herb	W	1	(Chen and Zhang, 2014)
40	<i>Cuminum cyminum</i>	Apiaceae	Herb	S	3	(Bouhlali et al., 2016; Tomy et al., 2014; Wei et al., 2015)
41	<i>Alnus nepalensis</i>	Betulaceae	Tree	L	1	(Saxena et al., 2018)
42	<i>Amaranthus dubius</i>	Amaranthaceae	Herb	L	1	(Tufts et al., 2015)
43	<i>Brassica oleracea</i>	Brassicaceae	Herb	L	1	(Tufts et al., 2015)
44	<i>Cucurbita maxima</i>	Cucurbitaceae	Herb	L	1	(Tufts et al., 2015)
45	<i>Eriobotrya japonica</i>	Rosaceae	Shrub	F	1	(Tufts et al., 2015)
46	<i>Mangifera indica</i>	Anacardiaceae	Tree	L	1	(Tufts et al., 2015)
47	<i>Ocimum americanum</i>	Lamiaceae	Herb	F	1	(Tufts et al., 2015)
48	<i>Ocimum gratissimum</i>	Lamiaceae	Herb	F	1	(Tufts et al., 2015)
49	<i>Psidium guajava</i>	Myrtaceae	Shrub	L	1	(Tufts et al., 2015)
50	<i>Solanum scabrum</i>	Solanaceae	Herb	F	1	(Tufts et al., 2015)
51	<i>Vigna unguiculata</i>	Fabaceae	Herb	L	1	(Tufts et al., 2015)
52	<i>Vitex payos</i>	Verbenaceae	Tree	F	1	(Tufts et al., 2015)
53	<i>Zanthoxylum chalybeum</i>	Rutaceae	Tree	L	1	(Tufts et al., 2015)
54	<i>Allium sativum L</i>	Liliaceae	Herb	Bu	1	(Ndoye Foe et al., 2016)
55	<i>Allium cepa L</i>	Liliaceae	Herb	Bu	1	(Ndoye Foe et al., 2016)
56	<i>Drypetes gossweileri</i>	Euphorbiaceae	Tree	Sb	1	(Ndoye Foe et al., 2016)
57	<i>Pentadiplandra brazzeana Baill</i>	Capparidaceae	Shrub	R	1	(Ndoye Foe et al., 2016)
58	<i>Cymbopogon flexuosus</i>	Poaceae	Herb	W	1	(Han and Parker, 2017b)
59	<i>Rosa damascena</i>	Rosaceae,	Shrub	Fl		(Bouhlali et al., 2016)

Leaves = L, root = R, Stem Bark =SB, Fruits =F, Bark =B, Aerial = Ae, Flower = Fl, Stem =St, Rhizo = Rh, Bulbs = Bu, Seed = S, Berries = Be, Whole =W

3.3.3. *In vivo* wound healing studies

Characteristics of the studies

Fifteen *in vivo* wound healing studies were eligible for data extraction and the year of publication ranged from 2001 to 2019. Except for one study that investigated both anti-inflammatory and wound healing activities, all studies investigated wound healing as a health outcome category. All studies included were peer-reviewed full research articles, and were conducted in four countries: Ethiopia (n=9), India (n=5), Pakistan (n=1) and Cameroon (n=1).

In this model, 14 medicinal plant species, namely *Commiphora guidottii* chiov, *Achyranthes aspera*, *Rumex abyssinicus*, *Calpurnia aurea*, *Croton macrostachyus*, *Kalanchoe petitiiana*,

685 *Aloe megalacantha*, *Stereospermum kunthianum*, *Hibiscus micranthus*, *Lantana camara*,
686 *Clematis longicauda*, *Clematis burgensis*, *Lawsonia inermis* and *Azadirachta indica*,
687 belonging to 12 families, were investigated (Table 2).

688 All studied medicinal plant parts had voucher numbers and were authenticated by a botanist.
689 The most studied plants parts were leaves (n=9) and the rest were stem bark (n=2), rhizome
690 (n=1), leaf latex (n=1) and one plant constituent, oleo-gum-resin. Eleven studies used excision
691 and incision wound models to investigate wound contraction and the period of epithelization
692 and wound tensile strength, respectively, while three of them used only excision wound
693 models. Swiss albino rats and Swiss albino mice of both sexes were used for both incision and
694 excision models.

695 The age range of mice used for the studies was 6 – 11 weeks and 12 – 35 weeks for rats. All
696 studies reported positive and negative controls: they used 0.2 % (w/v) nitrofurazone ointment
697 and 1% silver sulfadiazine cream as positive control and simple ointment BP as negative
698 control. Ten studies used one-way analysis of variance (ANOVA) followed by Dunnett's test
699 for experimental data analysis, and one study used the Student t-test. All studies considered a
700 95% confidence interval and $p < 0.05$ value as statistically significant.

701 Except for the studies conducted by Hawaze et al., (2013), Mekonnen et al., (2013) and
702 Begashaw et al., (2017), all studies prepared plant extracts and essential oils in a simple
703 ointment base according to the British Pharmacopoeia (BP) using hard paraffin, cetostearyl
704 alcohol, white soft paraffin and wool fat. Then, plant materials were incorporated into the
705 simple ointment to make plant extract/essential oil-ointments of uniform consistency and
706 smooth texture.

707 All investigated medicinal materials showed significant ($p < 0.05$) wound contraction at a
708 concentration range of 5% - 10% (w/w) and reduced the period of epithelialization relative to
709 the negative controls. Gebrehiwot et al. (2015) reported that 4% essential oil and 5% resin from
710 *Commiphora aguidottii* Chiov in ointment base, applied topically on a daily basis to incision and
711 excision wounds assisted wound contraction significantly ($p < 0.05$) from day 8 to 16 as
712 compared to the negative controls. In this study, resin showed a significant ($p < 0.05$) difference
713 in tensile strength compared to the simple ointment-treated groups.

714 In another study, reported by Fikru et al., (2012), 2.5%, 5% and 10% (w/w) 80% methanol
715 extracts of *Achyranthes aspera* increased the percentage of wound contraction starting from
716 day 9, and complete wound healing was seen in rats treated for 21 days. Epithelization time

was also shortened for animals treated with 2.5%, 5% and 10% (w/w) methanol extracts. On the 21st day after treatment, the 5% and 10% plant extract-treated group showed a relatively well-organized epidermal layer, increased number of mature fibroblasts (fibrocytes) cells and high levels of neovascularization and epithelization. The total DNA content in wound tissue samples of animals treated with 2.5%, 5% and 10% (w/w) plant extracts were shown significantly ($p<0.05$) higher than the amount detected in the negative control groups (Fikru et al., 2012).

Rat incision wounds treated with ethanol extract of *Lawsonia inermis* demonstrated a significant increase in skin breaking strength, an increase in the level of hydroxyproline content, and greater aggregation of macrophages with fewer fibroblasts and collagen fibres (Shivananda et al., 2008). In a similar study, commercially formulated *Lawsonia inermis* demonstrated faster reduction in scar tissue compared to non-treated animals (Khaliq et al., 2018).

In a study by Maan et al., (2017), rats treated with 15% (w/w) aqueous extract of *Azadirachta indica* showed significant wound contraction after the 10th day, and higher hydroxyproline content was exhibited by aqueous, aqueous-ethanol (1:1) and ethanol extracts when compared to vehicle-treated animals. Aqueous, aqueous-ethanol (1:1) and ethanol extracts showed higher skin breaking strength, and complete epithelization was observed after the 14th day of treatment, comparable to the reference drug. Similarly, topical application of 5% w/w ointment of *Azadirachta indica* significantly increased the percentage of wound contraction and decreased epithelization time. In addition, the mean scar area was significantly decreased, increased fibro-collagenous tissue deposition, and increased ($p<0.01$) tensile strength of the test groups compared to the control groups, which was comparable to the standard drug.

Mulisa et al., (2015) studied the methanol extract (5% and 10% (w/w) in ointment base) of *Rumex abyssinicus*, showing a significantly increased wound contraction rate in mice compared to control. Time to epithelization was shorter by 23.1% for 10% ($p<0.001$), 22.1% for nitrofurazone ($p<0.001$) and 16.3% for 5% ($p<0.05$) compared to those treated with the ointment base. The percentage of hydroxyproline content increased by 102.2% with nitrofurazone ($p < 0.001$) followed by 74% with 10% extract ($p < 0.001$) and 31.6% with 5% extract ($p < 0.05$). The tensile strength was significantly increased by approximately 36.2% ($p < 0.05$), 57.2% ($p < 0.001$), and 79.1% ($p < 0.001$) with 5% and 10% of the extract and

nitrofurazone ointments, respectively, compared to controls treated with the ointment base (Mulisa et al., 2015).

Similarly, Ayal and Belay (2019) evaluated methanol extract, chloroform, ethyl acetate and aqueous fractions of *Calpurnia aurea* at two concentrations (5% and 10%) in mice. They reported that 10% (w/w) crude extract ointment showed significant ($p < 0.05$) wound contraction on day two post-wounding, comparable with the activity of nitrofurazone 0.2% ointment in mice. Starting from day 4, the 10% w/w crude extract ointment revealed a higher percentage of wound contraction than the standard. The percentage of wound contraction of 10% w/w ointment of the aqueous fraction was also significantly ($p < 0.001$) higher on most post-wounding days, except on day 16 ($p < 0.01$) compared to the negative control. The periods of epithelialization of mice treated with the 5% w/w and 10% w/w aqueous fractions and the standard ointments were significant ($p < 0.01$) compared to the negative control. The mice treated with 10% w/w crude extract ointment had the highest percentage of tensile strength.

In another study by Fikru et al., (2012), the 80% methanol extract (5% and 10% in ointment base) of *Croton macrostachyus* showed a significant ($p < 0.05$) difference on the 6th day after treatment when compared with the simple ointment-treated group in rats. Likewise, the test groups needed a significantly shorter epithelization time. The 10th day photographs showed that the group treated with 10% (w/w) extract of *Croton macrostachyus* ointment demonstrated relatively better healing, with skin colour close to normal, and a smooth surface. Rats treated with 5 and 10% (w/w) ointments of the extracts showed higher breaking strengths (Mechesso et al., 2016).

Leaf latex of *Aloe megalacantha* (5% and 10 % in ointment base) were shown to have more rapid wound closure than standard drugs in albino-Wistar rats. The maximum rate of wound contraction was observed on the 11th (94.4%), 13th (100%), and 15th (100%) days after wound creation. The rate of wound closure was comparable with the 11th (88.58%), 13th (99.42%), and 15th (100%) treatment days when 0.2% nitrofurazone was used. The 5% (w/w) latex showed slightly more rapid epithelialization than either 10% (w/w) latex or reference drug-treated animals (Gebremeskel et al., 2018).

Mekonnen et al., (2013) studied 80% methanol extract, chloroform, methanol and distilled water fractions of *Kalanchoe petitiiana* in ointment bases and extracts and fractions alone in an excision and incision mouse wound model. Topical application of both 5% and 10% of the crude hydroalcoholic extract ointments to the excision wound model significantly increased (p

< 0.01) wound contraction rates and shortened the epithelization time compared to the control animals. The 5% ointment showed a slightly higher rate of wound contraction and shorter epithelization period than the higher dose (10% ointment). The chloroform fraction was devoid of any wound healing activity. The epithelization period was significantly shorter in animals treated with 5% and 10% concentrations of methanol and aqueous fractions ($p < 0.05$ in all cases) compared to controls. The hydroalcoholic crude extract showed greater wound healing effects than the three fractions, as evidenced by faster wound contraction rate and shorter epithelization period (Mekonnen et al., 2013) (Supplementary table 2).

The remaining three studies, namely Begashaw et al., (2017), Dash et al., (2017) and Hawaze et al., (2013) showed that methanol and aqueous extracts of *Hibiscus micranthus*, *Lantana camara*, *Clematis longicauda*, and *Clematis burgensis* exhibited significantly faster wound contraction, faster tissue epithelization, and significantly increased tensile strength of tissues in excision and incision wounds of rats (Supplementary table 2).

All the *in vivo* wound healing studies generated and applied an allocation sequence adequately. The distribution of relevant baseline characteristics was similar for the intervention and control groups. The timing of disease induction was adequate as per the protocol, and the allocation of laboratory animals to the different groups was properly concealed. However, six studies did not report whether they placed the cages or animals randomly within the animal room or facility (risk of performance bias). Five authors did not report the age category, and three authors did not report the sources of the laboratory animals. Two authors did not report the housing conditions of laboratory animals before or during the experiment, or the sources of diet for the animals. In addition, it was not clear whether the caregivers or investigators were blinded from knowing which intervention each animal received during the experiment, or whether the animals were selected at random or all animals were included in outcome assessment (risk of detection bias).

All studies reported that they used a study protocol that was available, and all the study's pre-specified primary and secondary outcomes were reported in the current manuscript. They used proper statistical analysis methods that were free of unit of analysis errors and free of design-specific risks of biases.

3.3.4. *In vitro* wound healing

Characteristics of the studies

Two *in vitro* wound healing studies were included. These were conducted in Ethiopia and Denmark. Both were peer-reviewed full articles, met the inclusion criteria, and were conducted according to internationally acceptable guidelines/protocols. The assay methods used for these *in vitro* tests were wound healing scratch assays, and antioxidant and radical scavenging activity assays, which were correlated with wound healing activity.

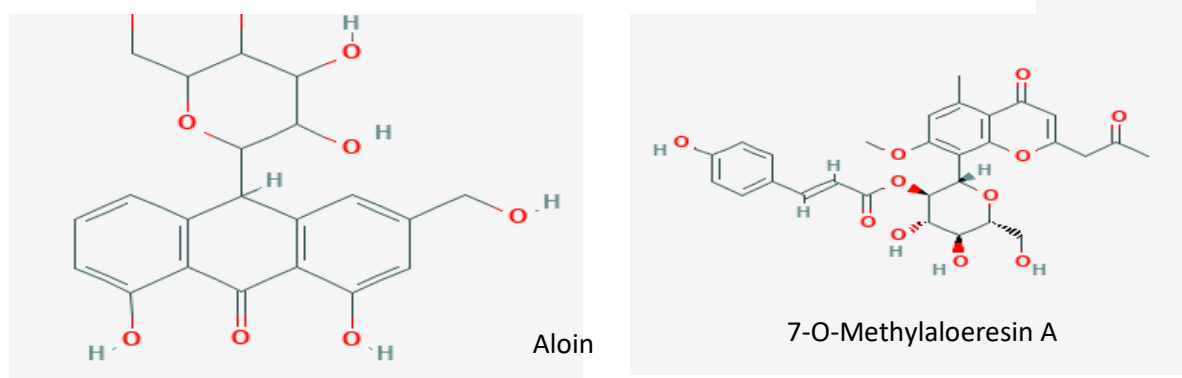
A total of eighteen Ethiopian medicinal plants were studied in the included two studies: *Aframomum melegueta*, *Allophylus spicatus*, *Annona senegalensis* Pers, *Folium Cissusqua drangularis*, *Herba Gymnanthemum coloratum*, *Folium cum Flos Radix*, *Jasminum dichotomum*, *Folium Leonotis nepetifolia*, *Melanthera scandens*, *Herba Millettia thonningii*, *Ocimum gratissimum* L., *Philenoptera cyanescens*, *Fructus Rourea coccinea*, *Folium Radix Thonningia*, *Herba Trichilia monadelpha*, *Triumfetta rhomboidei*, *Uvaria ovata* and *Aloe Harlan* (Table 2). These belong to 14 plant families. All the plants were authenticated by a botanist and had voucher numbers (Table 2).

Stamen, radix, aerial parts, leaves, cortex, leaf latex, stems and flowers bulbs were parts of the plants included in these *in vitro* studies; and leaf latex of aloe, aqueous, methanol, 70% ethanol, petroleum ether, ethyl acetate and methanol extracts were the types of plant extracts used in these studies.

Seventeen medicinal plants were evaluated at a concentration of 10 µg/ml for the *in vitro* wound healing. Only five of the 17 plant species tested increased proliferation or migration of fibroblasts in the scratch assay. These were *Allophylus spicatus* (warm and cold extracts of herbals), *Philenoptera cyanescens* (warm extract), *Melanthera scandens* (warm extract), *Ocimum gratissimum* (cold extract), and *Jasminum dichotomum* (warm extract). The warm water extract of folium/fructus of *Philenoptera cyanescens* was shown to have a 100% proliferation/migration rate compared to the negative control group (Freiesleben et al., 2017) (Supplementary table 2).

838 In the second study, the antioxidant activities of the latex and isolated compounds of *Aloe*
839 *harlana* Reynolds were tested using two complementary test systems, namely DPPH and 2-
840 deoxyribose degradation assay methods. It was shown that increasing concentrations (1000,
841 500, 250 and 125 µg/ml) of the latex and isolated compounds increased the percent radical
842 scavenging rates (Figure 2). The IC₅₀ of the latex was found to be 14.21 µg/ml, while that of
843 ascorbic acid (used as a reference) was 4.76 µg/ml. Both the isolated compounds and latex
844 exhibited free radical scavenging properties in a concentration-dependent manner. Similarly, a
845 compound, 7-O-Methylaloesresin A, was shown to have much stronger radical scavenging
846 activity (IC₅₀ = 0.021 mM) than aloin. At a concentration of 1.81 mM, 7-O-methylaloesresin A
847 was shown to have a maximum inhibitory effect of 63.4%, which was comparable to the
848 reference material, butylhydroxytoluene (BHT) (70.7%). Aloin was shown to have less activity
849 (IC₅₀ = 0.600 mM) in the deoxyribose degradation assay (Asamenew et al., 2011)
850 (Supplementary table 2).

Figure 2: Structures of aloin and 7-O-methylaloesresin A.



851

852 Both *in vitro* studies fulfilled the test definition criteria, the purpose and scientific basis of the
853 tests. In addition, valid and international acceptable *in vitro* method(s), written and documented
854 *in vitro* method description and related standard operating procedure(s) were followed.
855 However, neither study reported evidence of provision of adequate education and training for
856 personnel to promote high quality work and safety. In addition, there was no evidence of
857 minimisation of the risk of errors (e.g., mix-ups) or avoidance of cross-contamination which
858 might adversely affect the quality of the work performed, or safety measures to avoid
859 environmental and individual contamination. Furthermore, there was no report of equipment
860 calibration, or compliance of laboratory suppliers with GLP principles of international
861 standards which are recommended for formal quality systems.

Evidence showed that the cell lines were free from any contaminants, and that identity, functionality and genetic stability, batch number and sources of antioxidant test kits, media and cell lines were reported in both studies. Reference and control items were described well. However, no justification was provided for the selection of the reference item(s) or the applicability domains of the *in vitro* method, and limitations of the *in vitro* test were not well described. In addition, compatibility and toxicity of the solvent was not reported in either study.

Concentration levels of plant extracts and controls, number of replicas of experiments, evidence of uniform fashion treatment, cell seeding and reagents across the whole plate (well-to-well) were reported in both studies to minimise systematic errors.

Table 2: Summary of common medicinal plants identified from literature search for wound healing

S/N	Plant species	Family	Life form	Parts used	Number of citations	Ref.
1	<i>Commiphora guidottii</i> Chiov	Burseraceae	Tree	oleo-gum-resin	1	(Gebrehiwot et al., 2015)
2	<i>Achyranthes aspera</i>	Amaranthaceae	Herb	L	1	(Fikru et al., 2012)
3	<i>Rumex abyssinicus</i>	Polygonaceae	Herb	Rh	1	(Mulisa et al., 2015)
4	<i>Calpurnia aurea</i>	Fabaceae	Shrub	L	1	(Ayal and Belay, 2019)
5	<i>Croton macrostachyus</i>	Euphorbiaceae	Tree	L	1	(Mechesso et al., 2016)
6	<i>Kalanchoe petitiiana</i>	Crassulaceae	Herb	L	1	(Mekonnen et al., 2013)
7	<i>Aloe megalacantha</i>	Xanthorrhoeaceae	Shrub	L	1	(Gebremeskel et al., 2018)
8	<i>Stereospermum kunthianum</i>	Bignoniaceae	Herb	Sb	1	(Tsala, 2016)
9	<i>Hibiscus micranthus</i>	Malvaceae	Herb	L	1	(Begashaw et al., 2017)
10	<i>Lantana camara</i>	Verbenaceae	Herb	L	1	(Dash et al., 2001)
11	<i>Clematis longicauda</i>	Ranunculaceae	Shrub	L	1	(Hawaze et al., 2013)
12	<i>Clematis burgensis</i>	Ranunculaceae	Shrub	L	1	(Hawaze et al., 2013)
13	<i>Lawsonia Inermis</i>	Lythraceae	Tree	L	2	(Khaliq et al., 2018; Nayak BS, Isito G, Davis EM, 2008)
14	<i>Azadirachta Indica</i>	Meliaceae	Tree	L	2	(Maan et al., 2017; Nagesh et al., 2015)
15	<i>Aframomum legueta</i>	Zingiberaceae	Herb	S	1	(Freiesleben et al., 2017)
16	<i>Allophylus spicatus</i>	Sapindaceae	Tree	R	1	(Freiesleben et al., 2017)
17	<i>Annona senegalensis</i> Pers	Annonaceae	Shrub	L	1	(Freiesleben et al., 2017)

18	<i>Cissus quadrangularis folium</i>	<i>Vitaceae</i>	Herb	Ae	1	(Freiesleben et al., 2017)
19	<i>Gymnanthemum coloratum</i>	<i>Asteraceae</i>	Tree	L, Fl, R,	1	(Freiesleben et al., 2017)
20	<i>Jasminum dichotomum</i>	<i>Fabaceae</i>	Shrub	L	1	(Freiesleben et al., 2017)
21	<i>Leonotis nepetifolia</i>	<i>Oleaceae</i>	Herb	Ae	1	(Freiesleben et al., 2017)
22	<i>Melanthera scandens</i>	<i>Lamiaceae</i> ,	Herb	Ae	1	(Freiesleben et al., 2017)
23	<i>Millettia thonningii</i>	<i>Asteraceae</i>	Tree	B	1	(Freiesleben et al., 2017)
24	<i>Ocimum gratissimum</i> L	<i>Fabaceae</i>	Herb	Ae	1	(Freiesleben et al., 2017)
25	<i>Philenoptera cyanescens</i>	<i>Labiatae</i>	Shrub	L, F	1	(Freiesleben et al., 2017)
26	<i>Thonningia sanguinea</i> Vahl	<i>Connaraceae</i>	Herb	Ae	1	(Freiesleben et al., 2017)
27	<i>Trichilia monadelpha</i>	<i>Balanophoraceae</i>	Tree	B	1	(Freiesleben et al., 2017)
28	<i>Triumfetta rhomboidea</i>	<i>Meliaceae</i>	Shrub	R	1	(Freiesleben et al., 2017)
29	<i>Uvaria ovata</i>	<i>Annonaceae</i>	Shrub	B, R	1	(Freiesleben et al., 2017)
30	<i>Aloe harlana</i>	<i>Aloaceae</i>	Shrub	L	1	(Asamenew et al., 2011)

Leaves = L, root = R, Stem Bark =SB, Fruits =F, Bark =B, Aerial = Ae, Flower = Fl, Stem =St, Rhizo46me = Rh, Bulbs = Bu, Seed = S, Berries = Be, Whole =W

4. Discussion

The purpose of this review was to demonstrate the anti-inflammatory and wound healing activities of Ethiopian medicinal plants that might potentially be used for limb care (particularly, tropical lymphoedema and associated wounds). This systematic review identified a total of 63 articles covering four different experimental models. Of these, 31 used *in vivo* anti-inflammatory, 15 *in vitro* anti-inflammatory, 15 *in vivo* wound healing, and 2 *in vitro* wound healing models. Overall, medicinal plant extracts tested for these two conditions in *in vitro* and *in vivo* models were shown to have good activity. Despite the heterogeneity of the studies, all plant extracts investigated succeeded in reducing experimentally-induced inflammation in animals, in down-regulation of inflammatory and pro-inflammatory cells, and in promotion of wound healing.

In *in vivo* anti-inflammatory assays, 80% methanol root extract of *Jasminum abyssinicum* was shown to have good activity in a dose-dependent manner, possibly through secondary metabolites such as saponins, terpenoids, triterpenes, glycosides and flavonoids (Tadiwos et al., 2017). The anti-inflammatory activity might also be attributed to polyphenols like flavonoids, which are present in the extracts and exert their activity through free radical scavenging and inhibition of inflammatory pathways (Kaushik et al., 2012). This agrees with reports that flavonoids inhibited the biosynthesis of prostaglandins by inhibiting COX and LOX inflammatory pathways (Adebayo et al., 2015; Amoo et al., 2012).

Acetone extract of *Vernonia amygdalina* (Gerawa) was also shown to have fast activity at low concentrations due to its inhibitory effect on the biosynthesis of prostaglandin E2 (PGE2) and prostaglandin D2 (PGD2). It contains secondary metabolites such as tannins, potent cyclooxygenase inhibitors (Adedapo et al., 2014). Tannins also act on leukocyte migration and antagonism of the phlogistic actions of mediators of inflammation (M.L.R. Mota, G. Thomas, 1985). This report is in agreement with the findings of Mulisa and colleagues (Mulisa et al., 2015).

In another study, aqueous extracts of the root and stem of *Citrullus colocynthis* were shown to have good activity compared to aspirin. This activity is due to the presence of secondary metabolites (alkaloids), which have analgesic and anti-inflammatory activities and act via inhibition of pro-inflammatory cytokines (IL-6 and IL-1) and the expression of COX-2, and increasing the level of anti-inflammatory cytokine IL-4 in carrageenan-injected rat paw tissues (Marzouk et al., 2010). A similar mechanism of action of alkaloids was reported by others (Bribi et al., 2015). The anti-inflammatory and analgesic properties of alkaloids have also been reported by other authors (Barbosa-Filho et al., 2006; Chester et al., 2017). For instance, colchicine is an alkaloid used for the treatment of gout (arthritis) and leukocytoclastic vasculitis (Barbosa-Filho et al., 2006).

The methanol extract of *Leonotiso cymifolia* (Alemu et al., 2018), aqueous extract of *Myrtus communis* (Hosseinzadeh et al., 2011), ethanol and aqueous extract of *Caesalpinia pulcherrima* (Sharma and Rajani, 2011) were evaluated for chronic inflammation and showed inhibition of formation of inflammatory exudates and granuloma mass in a dose-dependent manner compared to the standard drugs. Aqueous extract of *Caesalpinia pulcherrima* (200 mg/kg dose) showed better activity than indomethacin (Sharma and Rajani, 2011) because of its major

constituent, flavonoids, known to inhibit fibroblasts and the synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Rao et al., 2005).

The essential oil of *Eugenia caryophyllata* and its main active component, eugenol (Figure 3), were shown to have anti-inflammatory and pro-wound healing activity in an *in vitro* model due to the significant inhibition of many pro-inflammatory cytokines in dermal fibroblast cells (Han and Parker, 2017a). In another study, eugenol was shown to have a similar anti-inflammatory activity to the COX antagonist (indomethacin) and COX-2 selective antagonist (celecoxib) in a similar model (Daniel et al., 2009). 0.01% essential oil of *Cuminum cyminum* exhibited prominent anti-inflammatory activity by inhibiting inducible NO synthase (iNOS) and cyclooxygenase (COX-2) mRNA expression in LPS-stimulated RAW 2647 cells. Besides, 0.01% essential oil of *Cuminum cyminum* decreased the levels of IL-1 and IL-6, which are important factors in inflammatory responses and chronic inflammatory diseases (Wei et al., 2015).

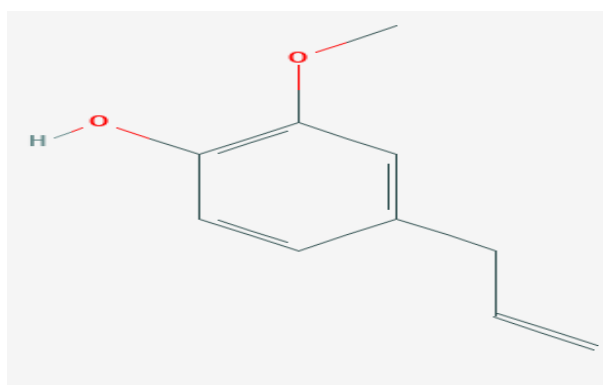


Figure 3: Structure of eugenol

Citral, the main component of the essential oil of lemongrass, inhibits the production of TNF- α , IL-8, VCAM-1, and ICAM-1 in human umbilical vein endothelial cells and promotes wound healing through a tissue remodelling process (Song et al., 2016). The effects of *Allium cepa* and *Pentadiplandra brazzeana* essential oils were found to be 2 times greater than those of sodium diclofenac against heat denaturation of bovine serum albumin (BSA) (Foe et al., 2016).

Lawsonia inermis was shown to have anti-inflammatory activity in *in vivo* and *in vitro* studies, (Bouhlali et al., 2016; Manivannan and Aeganathan, 2015; Chaibi et al., 2017). Flavonoids isolated from *Lawsonia inermis* reduced carrageenan-induced paw oedema and inflammation due to their antioxidant activity and their ability to modulate gene expression of cytokines and adhesion molecules (Castell, 2016). Similarly, the methanol extract of *Lawsonia inermis* and two alkaloids isolated from the methanol extract inhibited lipo-oxygenase enzyme by

decreasing the production of nitric oxide (NO) and pro-inflammatory cells and suppressing the biosynthesis of prostaglandins (Chaibi et al., 2017). Furthermore, *Lawsonia inermis* was reported to inhibit thermal induced tissue protein denaturation and promoted membrane stability by altering the electrostatic bond of hydrogen, and hydrophobic and disulphide bonds (Annavarapu et al., 2016).

Similarly, *Azadirachta indica* was reported to have significant anti-inflammatory activity in both *in vivo* and *in vitro* studies. The chloroform extract and fractions reduced carrageenan-induced oedema and granulation tissue formation in rats more powerfully than indomethacin. In addition, the fractions inhibited pro-inflammatory cells (IL-1 and TNF- α), COX-1 and COX-2 in *in vitro* tests by inhibiting the interaction between pathogen-associated molecular patterns (PAMPs) and toll receptors (TLRs), which impair the expression and production of pro-inflammatory cells (Umar et al., 2014). Similarly, seed oil of *Azadirachta indica* inhibited oedema formation after carrageenan injection. The phytochemical constituents of the oil, flavonoids, are shown to inhibit prostaglandin biosynthesis, endoperoxides, enzyme-like protein kinase and phosphodiesterase, which have key roles in inflammation (Naik et al., 2014).

Bhosale *et al.* (2012) reported the anti-inflammatory activity of aqueous extract of *Achyranthes aspera* in carrageenan-induced paw oedema mice, which was attributable to flavonoids, alkaloids, saponins, and triterpenoids phytoconstituents found in the extracts. Similarly, Gokhale et al., (2002) reported anti-inflammatory activity of the ethanolic extract of *Achyranthes aspera* in Wistar rats due to the aforementioned plant constituents.

For the *in vivo* anti-inflammatory assay, the most frequently used experimental model was carrageenan-induced paw oedema. This is a highly sensitive and reproducible test for non-steroidal anti-inflammatory drugs and is established as a valid and widely used model to study new anti-inflammatory drugs and mechanisms of action (Dyzoyem and Bakowsky, 2017; Posadas et al., 2004). It is a useful method for detecting orally active anti-inflammatory agents and has significant predictive value for anti-inflammatory agents acting through mediators of acute inflammation (Dyzoyem and Bakowsky, 2017). It induces a biphasic oedema in the mouse paw. The first phase (0-1h) is characterized by an acute and local inflammatory response which involves secretion of histamine, serotonin, and bradykinin. The second phase usually develops after 24h, and is characterized by more pronounced oedema with maximum effect in 48-72h. Prostaglandins and cytokines (IL-1 β , IL-6, IL-10, and TNF- α) are inflammatory mediators involved in the second phase of oedema (Dyzoyem and Bakowsky,

2017; Morris, 2003; Posadas et al., 2004). The inflammatory response of carrageenan was quantified by measuring the circumference of oedema, which is maximal around 5h post-carrageenan injection (Morris, 2003).

ELISA was used for the *in vitro* anti-inflammatory assay to measure inflammatory biomarkers such as cytokines, chemokines, and metalloproteinases produced in the supernatants in LPS-stimulated cell culture. ELISA uses antibodies to differentiate epitopes of cytokines and uses them to capture and quantify an analyte of interest (cytokines from a cell culture supernatant) with a remarkable sensitivity (Chiswick et al., 2012; Rosengren et al., 2003). Similarly, Western blotting and gene expression quantification were used to measure the gene responsible for the production of inflammatory biomarkers.

Wound healing is a complex process that includes a harmonized interaction between different immunological and biological systems (Velnar et al., 2009). It is divided into four phases. These are haemostasis, inflammation, proliferation and tissue remodelling, which are overlapping processes in time (Li et al., 2007; Singh et al., 2017). The first phase involves blood coagulation, haemostasis, and cellular events such as infiltration of leukocytes with different purposes in antimicrobial and cytokine release, which initiates the proliferative response for wound repair (Li et al., 2007). The second phase of wound healing is inflammation, whose aim is to prevent infection (Singh et al., 2017). The third phase is characterized by fibroblast migration and deposition of newly synthesized extracellular matrix, acting as a replacement for the provisional network composed of fibrin and fibronectin. Finally, the remodelling phase is responsible for the development of new epithelium and final scar tissue formation (Velnar et al., 2009).

Plant extracts have been reported for their wound healing activity, and promote wound healing through angiogenesis, activation of NF- κ B, favouring pro-inflammatory cytokines, upregulation of iNOS and alpha-1 type-1 collagen, fibroblast proliferation, and anti-oxidant activity (Firdous, 2018).

The wound healing studies reported in this review tested the ability of plant extracts and essential oils to contract wounds, reduce the period of epithelization, increase the tensile strength of the wound (Dash et al., 2001; Tsala, 2016), influence cell migration and proliferation (Freiesleben et al., 2017), and act through antioxidant and anti-microbial activities (Asamenew et al., 2011), leading to tissue repair and restoration of function.

In the study reported previously, the oil and resin of *Commiphora aguidottii* increased wound contraction in rats, enhanced the proliferation of epithelial cells in incision wounds and increased tensile strength in mice. The resins and oil were shown to increase collagen synthesis as well as aid the cross-linking of proteins (Gebrehiwot et al., 2015).

In another study, incision and excision wounds of laboratory animals treated with 5% and 10% methanol extracts of *Achyranthes aspera* showed faster healing, increased cellular proliferation and shorter epithelization time in albino rats, which could be due to stimulation of cellular proliferation and enhancement of collagen synthesis (Fikru et al., 2012). This report is in agreement with the study reported by Barua et al., (2012) and Edwin et al., (2008) which indicated that methanol and water extracts of *A. Aspera* promoted wound healing, likely due to the secondary metabolites, triterpenes, known to promote wound healing process through their astringent and antimicrobial property. Tannins promote capillary vasoconstriction, which decreases vascular permeability and causes a local anti-inflammatory effect (Edwin et al., 2008). Phytochemical screening of the extracts revealed the presence of tannins and triterpenes in the water and methanol extracts of *A. Aspera* (Barua et al., 2012; Edwin et al., 2008).

Mulisa and colleagues tested 4% and 10% (w/w) methanol extract (in ointment base) of *Rumex abyssinicus* which significantly increased the percent wound closure in excision wounds; shortened the epithelization time and increased the hydroxyproline content (Mulisa et al., 2015). In another study, *Rumex abyssinicus* was shown to possess antibacterial and anti-inflammatory activities; and the ability to regenerate epithelial cells, which might contribute to wound healing (Getie et al., 2003).

Ointments prepared from a solvent fraction of *Calpurnia aurea* were shown to reduce the epithelization time and increase wound contraction in mice, which might be attributed to the secondary metabolites present in the fraction, such as terpenoids, tannins, alkaloids, saponins, phenols and flavonoids that are known to promote wound healing via anti-bacterial and anti-inflammatory activities (Ayal et al., 2019). In the rat excision wound model, 10% ointment of methanol extract of *Croton macrostachyus* showed the fastest and most complete wound healing compared with the negative control. This activity may be due to its individual activity or the combined activity of phytoconstituents such as anthraquinones, flavonoids, phytosterols, polyphenols, saponins and tannins (Abraham et al., 2016).

The wound healing effect of the latex of *Aloe megalacantha* in an ointment form in excision and incision wound models in rats was greater than that of the standard drug, nitrofurazone,

which might be attributable to an individual or multiple phytochemical constituents (Gebremeskel et al., 2018). This finding is consistent with other reports of wound healing activity of other species of Aloe (Asamenew et al., 2011; Dat et al., 2014). Similarly, the hydroalcoholic extract of *Kalanchoe petitiiana* in an ointment base was shown to have better wound healing activity than 0.2% nitrofurazone ointment.

A delay in wound healing was observed at higher concentrations of the extracts. There was an enhanced rate of wound contraction and a reduction in healing time in animals treated with ointment containing the methanol and aqueous fractions of *Kalanchoe petitiiana*. The tensile strength of 5% and 10% ointments of the crude extract treated groups were slightly greater than those of the 0.2% nitrofurazone group. These activities might be due to the phytoconstituents, especially flavonoids, which play a great role in wound healing activity (Mekonnen et al., 2013).

The wound healing activity of *Lawsonia inermis* shown that henna leaf extracts had a high healing rate due to secondary metabolites (Khaliq et al., 2018; Nayak et al., 2008). Similar, stem bark extracts of *Azadirachta indica* was shown to have a high level of hydroxyproline and increased total protein content, which are indicators of wound healing. In addition, it increased wound contraction, and DNA content in the scar tissue, which was a sign of cellular proliferation (Maan et al., 2017).

Allophylus spicatus, *Philenoptera cyanescens*, *Melanthera scanden*, *Ocimum gratissimum*, and *Jasminum dichotomum* were reported to increase proliferation and migration of cells in 3T3 fibroblasts cell lines (*in vitro*). The warm water extract of folium/fructus of *Philenoptera acyanescens* was shown to have the highest (100%) proliferation and migration rate compared to the negative control groups, which was attributable to the active compounds rutin (quercetin-3-O- rutinoside) and quercetin-triglycoside, isolated from *Philenoptera cyanescens*. Both compounds have anti-oxidant properties and are known in promoting cell proliferation (Freiesleben et al., 2017). Figure 4.

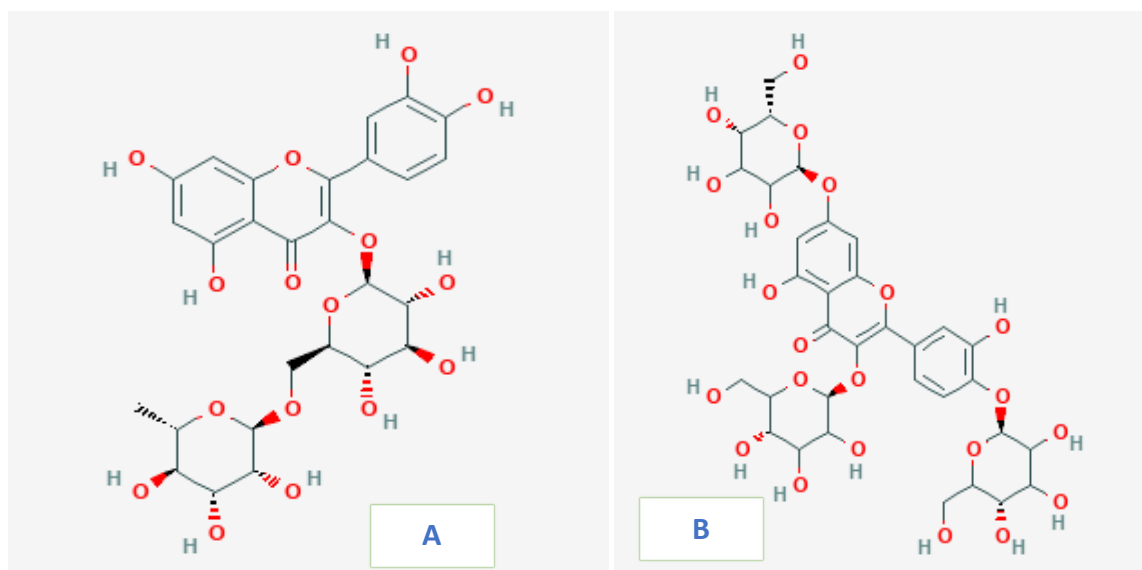


Figure 4: Chemical structure of quercetin-3-O- rutinoside (A) and quercetin- 3,7,4' -triglycoside (B)

The antioxidant properties of *Aloe harlana* Reynolds were evaluated using two assay methods, DPPH and deoxyribose degradation assay. The latex and the compounds isolated from *Aloe harlana* Reynolds correlated with the traditional use of the plant for the treatment of wounds, infection and inflammatory diseases (Asamenew et al., 2011). Antioxidants are assumed to help in controlling wound oxidative stress, which accelerates wound healing. They are important mediators in regulating the damage that is potentially incurred by biological molecules such as DNA, protein, lipids, and body tissue in the presence of reactive species (Barku, 2019).

For the *in vivo* wound healing model, excision and incision wound models were the most frequently used methods to test wound contraction, period of epithelization and wound tensile strength. The wound breaking strength was measured using the incision method while collagen estimation, period of epithelization and wound contraction were measured using the excision method (Gautam et al., 2014). Among the *in vitro* wound assay methods, the wound scratch assay was the most frequently used method and used alongside well-developed methods to measure cell migration *in vitro*. A “wound gap” in a cell monolayer is created by scratching, and the “healing” of this gap after treatment with plant extracts such as cell migration and growth towards the centre of the gap is monitored and quantified (Chen, 2012). This is particularly suitable for studies on the effects of cell interactions on cell migration, which mimic *in vivo* cell migration and are compatible with imaging of live cells during migration to monitor intracellular events (Liang et al., 2007).

5. Conclusion

From the present study, it can be concluded that there are promising medicinal plant extracts and compounds traditionally employed in Ethiopia that might be used as anti-inflammatory and wound healing agents. Among these plants, *Lawsonia inermis*, *Azadirachta indica*, *Cuminum cyminum* and *Achyranthes Aspera* are the most studied plant species for the management of wound infection and inflammation, whereas *Lawsonia inermis* and *Azadirachta indica* were the most commonly studied for wound healing.

In terms of effectiveness, methanol extract of *Jasminium abyssinicum*, chloroform extract of *Azadirachta indica*, a flavonoid isolated from *Lawsonia inermis* and acetone extract of *Vernonia amygdalina* are the most effective anti-inflammatory agents in animal models. Similarly, methanol extracts of *Lawsonia inermis*, essential oil of *Eugenia caryophyllata*, butanol fraction of *Alnus nepalensis*, *Amaranthus dubius*, *Ocimum americanum*, *Vigna unguiculata*, and *Zanthoxylum chalybeum* are the most effective plant extracts in regulating inflammatory and pro-inflammatory cells in *in vitro* models. On the other hand, ethanol extract of *Lawsonia inermis*, aqueous extract of *Azadirachta indica*, 5% w/w ointment of *Azadirachta indica*, extract and fractions of *Calpurnia aurea* in 10% ointment base, 10% (w/w) extract of *Croton macrostachyus* in ointment base, leaf latex of *Aloe megalacantha* (5% and 10% in ointment base) are the most effective plant materials for the management of wounds in animal models.

6. Implications for future research and recommendations

It is vital to systematically summarize, and document medicinal plants tested against a range of diseases and used traditionally for treatment, and to test further their effectiveness against a range of disease-related pathologies such as lymphoedema. Information about many medicinal plants is fragmented, meaning that systematic compilation and synthesis are important. This systematic review helped identify medicinal plants for the planned research aimed to develop safe, effective and affordable alternatives for the management of tropical lymphoedema. It will also create an opportunity for future research and practice by identifying and characterizing compounds that could be developed into new standardized medicines. Medicinal plants hold much promise for treating diseases, and there is a great potential for new drug discovery and development.

Abbreviations/Acronyms

1122	ANOVA - one-way analysis of variance
1123	ARRIVE - Animal Research: Reporting of <i>In Vivo</i> Experiments
1124	BHT – Butylhydroxytoluene
1125	CAMARADES - Collaborative approach to Meta-Analysis and Review of Animal Data from
1126	Experimental Studies
1127	CC ₅₀ - Minimum dose that is toxic to 50 % of cells
1128	CD4 - cluster of differentiation 4
1129	CFU - Colony forming unit
1130	Chi ² test – Chi square test
1131	CI - Confidence interval
1132	COX - Cyclooxygenase
1133	DLA – dermatolymphangioadenitis
1134	DMSO - Di-methyl-sulfoxide
1135	DNA – Ribonucleic acid
1136	DPPH - 2,2-Diphenyl-1-picrylhydrazyl
1137	ED ₅₀ - Minimum dose that is effective to 50% of the population
1138	ELISA - enzyme-linked immunosorbent assay
1139	EMBASE- Excerpta Medical data BASE
1140	GBD - Global Burden of Disease
1141	GIVIMP - Guidance Document on Good <i>In-Vitro</i> Method Practices
1142	GLP - Good laboratory practice
1143	HDF3CGF- Primary human neonatal fibroblasts
1144	HEK293 - human embryonic kidney 293
1145	HMGB1- High mobility group box 1 protein
1146	I ² test- I square test statistics
1147	IC ₅₀ - half minimum inhibitory concentration
1148	IFN-γ – interferon gamma
1149	IL1 – Interleukin 1
1150	IL10 - Interleukin 10
1151	IL13 - Interleukin 13
1152	IL1β – Interleukin one beta
1153	IL4 - Interleukin 4

1154	IL6 - interleukin 6
1155	IL8 - Interleukin-8
1156	iNOS - Inducible nitric oxide synthase
1157	IP-10 - Interferon gamma-induced protein 10
1158	I-TAC - Interferon-inducible T-cell a chemoattractant
1159	ITN - Iodonitrotetrazolium Chloride
1160	JNK – Jun N-terminal kinase
1161	LD ₅₀ - Lethal dose in 50% of the population
1162	LD ₅₀ – Minimum dose that is lethal to 50% of the population
1163	LF - Lymphatic filariasis
1164	LOX - Lipoxygenase
1165	LPS- Lipopolysaccharides
1166	M2 - Macrophage type 2
1167	MeSH - Medical Subject Heading
1168	mg/ml – milli gram per ml
1169	MIC- Minimum inhibitory concentration
1170	MIG - monokine induced by gamma interferon
1171	MLD - Manual lymphatic drainage
1172	mRNA -Messenger Ribonucleic Acid
1173	NF-kB p65 – nuclear factor kappa-light-chain-enhancer of activated B cells
1174	NO - Nitric oxide
1175	NSAIDs - Non-Steroidal anti-inflammatory drugs
1176	NTD – Neglected Tropical Disease
1177	OECD - Organization for Economic Cooperation and Development
1178	PAMPs - pathogen- associated molecular patterns
1179	PBS- phosphate buffered saline
1180	PCR – Polymerase chain reaction
1181	PICO – Population, Intervention, Comparator, Out-come
1182	PRISMA-P - Preferred reporting items for systematic review and meta-analysis protocols
1183	PROSPERO - International prospective register of systematic reviews
1184	PUBMED/MEDLINE- National library of Medicine
1185	RAW264.7 cells lines

1186 RoB – Risk of bias
1187 SD – standard deviation
1188 SPSS – Statistical package for social science
1189 TGF-B1- transforming growth factor beta-1
1190 TLRs - toll receptors
1191 TM - Traditional medicine
1192 TNF-alpha – Tumour necrosis factor alpha
1193 TNF α - Interferon alpha
1194 VCAM-1 - vascular cell adhesion molecule 1
1195 WHO - World Health Organization
1196 ZI – zone of inhibition
1197

1198 **Author contributions:** DN, GD, EM, TB, MB, BAL and AF were involved in
1199 conceptualization and design of the study, and in collection, analysis and interpretation of the
1200 data. DN wrote the first draft of the manuscript and DN, GD, EM, TB, MB, BAL and AF
1201 critically reviewed the manuscript for intellectual content. All authors have read and approved
1202 the final manuscript.

1203 **Acknowledgments:** We would like to thank CDT-Africa, Addis Ababa University and
1204 Ethiopian Public Health Institute for organizing and sponsoring trainings on systematic review
1205 and meta-analysis; and my special gratitude to Clare Callow, Grit Gansch and Manuela
1206 McDermid for supporting us on work related to administration issues.

1207 **Funding:** This research was funded by the National Institute for Health Research (NIHR)
1208 Global Health Research Unit on NTDs at BSMS using UK aid from the UK Government to
1209 support global health research. The views expressed in this publication are those of the author
1210 (s) and not necessarily those of the NIHR or the UK Department for Health and Social Care.

1211 **Competing interests:** none of the authors has any conflict of interest regarding this study.

1212

1213 **Ethical approval:** not applicable

1214 7. References

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